Phenotypic screening identifies HDAC6 inhibitors as cardioprotective agents in BAG3 cardiac-knockout mouse model of dilated cardiomyopathy

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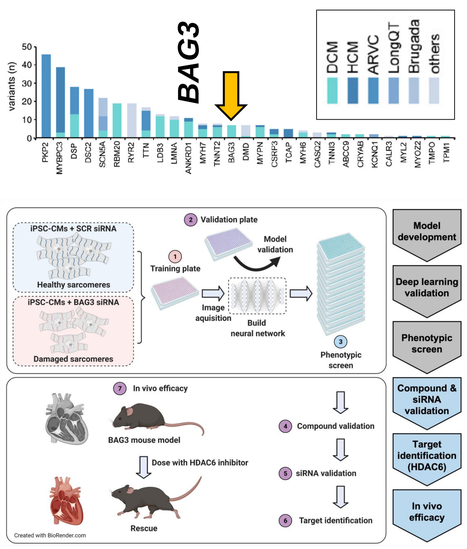
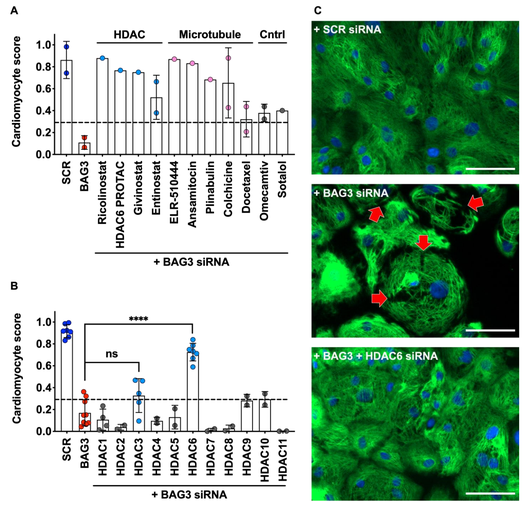
Use of iPSC-CM as Drug Discovery Platform For Dilated Cardiomyopathy



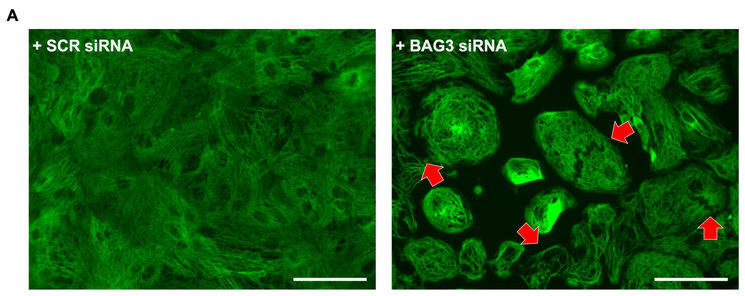
Background: Familial dilated cardiomyopathy (DCM) is characterized by reduced cardiac output, as well as thinning and enlargement of left ventricular chambers. These characteristics eventually lead to heart failure. Current standards of care do not target the underlying molecular mechanisms associated with genetic forms of heart failure, driving a need to develop novel therapeutics that target genetic DCM.
Purpose: To identify candidate therapeutics, we developed an in vitro DCM model using induced pluripotent stem cell–derived cardiomyocytes (iPSC-CMs) deficient in BAG3.
Methods and Results: Using BAG3-deficient iPSC-CMs, we identified cardioprotective drugs with a phenotypic screen and deep learning. Using a library of 5500 bioactive compounds and siRNA validation, we identified that inhibiting HDAC6 was cardioprotective at the sarcomere level. We translated this finding to a BAG3 cardiac-knockout ($BAG3^{cKO}$) mouse model of DCM, showing that inhibiting HDAC6 with two isoform-selective inhibitors (tubastatin A and a novel inhibitor TYA-018) protected heart function. HDAC6 inhibitors also protected the microtubule network from mechanical damage, increased autophagic flux, decreased apoptosis, and reduced inflammation in the heart.
Conclusion: HDAC6 inhibitors improved left ventricular ejection fraction and extended lifespan in a $BAG3^{cKO}$ mouse model of DCM. Our results demonstrate the power of combining iPSC-CMs with phenotypic screening and deep learning to identify therapeutic targets for DCM.

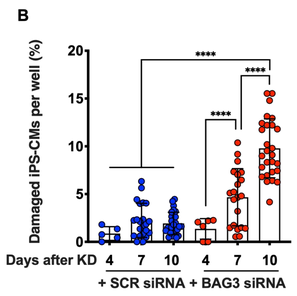
Target Validation Shows Inhibiting HDAC6 is Sufficient to Protect Against Sarcomere Damage



(A) Top compound classes (HDAC inhibitors, microtubule inhibitors) from the library screen and two cardiovascular standard-of-care agents [omecamtiv mecarbil (Omecamtiv) and sotalol] identified from the screen were validated at a 1µM dose using the cardiomyocyte score. Data from 1-2 independent biological replicates. n = 4 – 16 technical replicates per condition. Error bars = SD.
(B) Further validation using siRNAs showed that knockdown of HDAC6 protected against sarcomere damage in $BAG3^{KD}$ iPSC-CMs using the cardiomyocyte score with the deep learning algorithm. Data from 2-7 independent biological replicates. n = 4 – 16 technical replicates per condition. Error bars = SD. ****P < 0.0001.
(C) Representative immunostaining of anti-MYBPC3 in iPSC-CMs treated with scramble (SCR), BAG3, or BAG3+HDAC6 siRNA. Arrows indicate sarcomere damage. Scale bar = 50 µm.

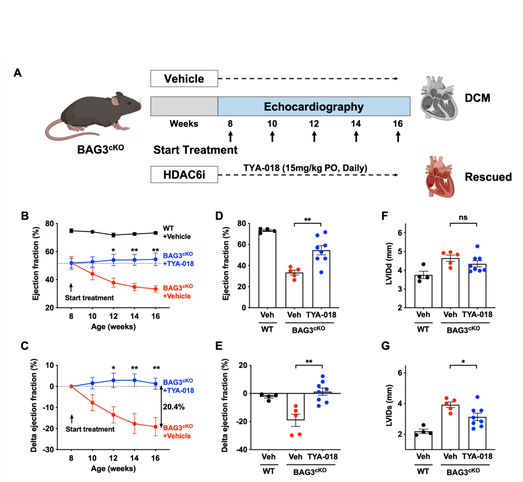
BAG3 Deficiency Leads to Sarcomere Damage





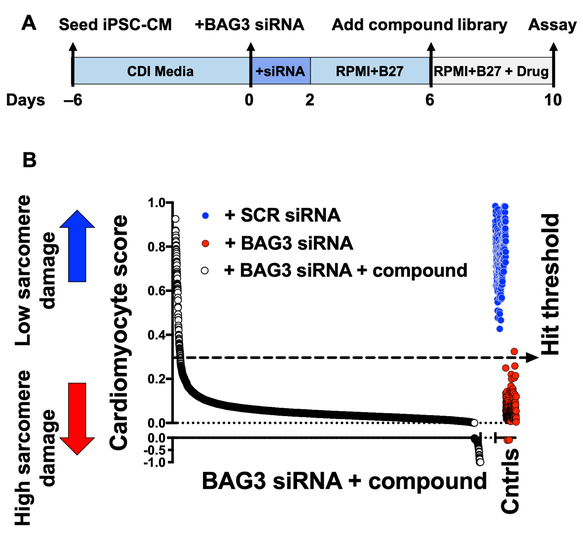
(A) Representative immunostaining of iPSC-CMs treated with SCR or BAG3 siRNA, and then stained with an antibody against MYBPC3. Arrows indicate breaks and reduced sarcomere content in BAG3 knockdown cells. Scale bars = 50 µm. (B) Quantification of sarcomere damage in iPSC-CMs treated with SCR or BAG3 siRNA. The number of damaged iPSC-CMs increased as a function of time in BAG3 knockdown (KD) cells. Error bars = SD. ****P < 0.0001.

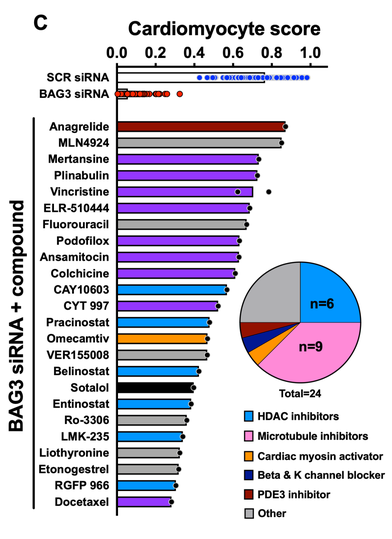
Inhibiting HDAC6 with TYA-018 Protects Heart Function in $BAG3^{cKO}$ Mice



(A) Schematic of drug treatment in $BAG3^{cKO}$ mouse model. TYA-018 (highly selective HDAC6 inhibitor) was administered daily by oral gavage at 15 mg/kg starting when mice were 2 months of age.
(B) Daily dosing of TYA-018 protected heart function during the 8-week dosing period as measured by ejection faction. Error bars = SEM. **P < 0.01.
(C) Ejection fraction was tracked from the first day of dosing, and delta ejection fraction was measured. During the 8-week period, heart function did not decline in the TYA-018-treated arm, whereas it dropped by 19.1% in the vehicle-treated arm. Error bars = SEM. **P < 0.01.
(D & E) Ejection fraction (D) and delta ejection fraction (E) (compared to the pre-dose baseline) at 4 months of age and 8 weeks of dosing shows TYA-018 protects against declining heart function in $BAG3^{cKO}$ mice. Error bars = SEM. **P < 0.01. Veh, vehicle.
(F & G) Left ventricular internal diameter at diastole (LVIDd) (F) and systole (LVIDs) (G) were reduced by TYA-018 in $BAG3^{cKO}$ mice, bringing the levels closer to that of their wild-type (WT) littermates. Error bars = SEM. *P < 0.05.

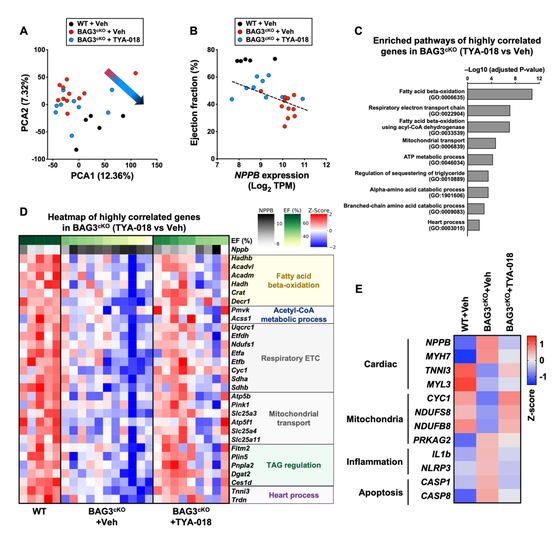
High throughput Screening Identifies HDAC and Microtubule Inhibitors a Cardioprotective Agents





(A) An unbiased screen was performed using a library of 5500 bioactive compounds. iPSC-CMs were treated with BAG3 siRNA and compounds at a concentration of 1µM. Hits were first identified using deep learning on control iPSC-CMs treated with either SCR or BAG3 siRNA. The hit threshold was set at a cardiomyocyte score of 0.3. (C) The top 24 compounds consisted of histone deacetylase (HDAC) and microtubule inhibitors. In addition, three known heart failure agents were identified: sotalol (beta-blocker and K-channel blocker), omecamtiv mecarbil (cardiac myosin activator), and anagrelide (PDE3 inhibitor).

TYA-018 Restores Dysregulated Cardiac and Metabolic Transcripts in $BAG3^{cKO}$ Hearts



(A) Hearts from all three arms of the study were analyzed using RNA-Seq. Principal component analysis of all coding genes (with Log_2 TPM >1) showed a global correction of $BAG3^{cKO}$+TYA-018 coding genes toward WT mice.
(B) RNA-Seq analysis shows *NPPB* expression increased by approximately fourfold in $BAG3^{cKO}$ mice compared to WT mice at 4 months of age. TYA-018 treatment reduced *NPPB* levels by twofold in $BAG3^{cKO}$ mice. The level of *NPPB* in $BAG3^{cKO}$+TYA-018 mice was anticorrelated with heart function.
(C) The top terms in the biological process category enriched in $BAG3^{cKO}$ mice treated with TYA-018. These genes were selected based on positive correlation (R>0.5) with cardiac function (EF%) and negative correlation (R<-0.5) with *NPPB* expression.
(D) Heatmap shows genes from the selected GO terms.
(E) Heatmap of RNA-Seq analysis from a selected number of genes. The data shows correction of key sarcomere genes (*MYH7*, *TNNI3*, and *MYL3*) and genes regulating mitochondrial function and metabolism (*CYC1*, *NDUFS8*, *NDUFB8*, *PPKARG2*) in $BAG3^{cKO}$+TYA-018 mice. Inflammatory (*IL-1β*, *NLRP3*) and apoptosis (*CASP1*, *CAPS8*) markers were also reduced.